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ABOUT COVER

Peer-reviewer of *World Journal of Clinical Cases*, Prof. Adrián Ángel Inchauspe, obtained his MD in 1986 from La Plata National University (Argentina), where he remained as Professor of Surgery. Study abroad, at the Aachen and Tubingen Universities in Germany in 1991, led to his certification in laparoscopic surgery, and at the Louis Pasteur University in Strasbourg France, led to his being awarded the Argentine National Invention Award in 1998 for his graduate work in tele-surgery. He currently serves as teacher in the Argentine Acupuncture Society, as Invited Foreigner Professor at the China National Academy of Sciences and Hainan Medical University, and as editorial member and reviewer for many internationally renowned journals. (L-Editor: Filipodia)

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ORIGINAL ARTICLE

Case Control Study

Impact of *mTOR* gene polymorphisms and gene-tea interaction on susceptibility to tuberculosis

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Author contributions: Wang M and Ma SJ contributed to data analysis and writing of the manuscript; Wu XY, Zhang X and Huang X contributed to the data collection, data analysis and discussion; Xiao ZH, Yan HP and Wang J contributed to the literature search, language editing, and manuscript revision; Abesig J and Tan HZ contributed to the language editing and manuscript revision; Chen MS contributed to the study design, manuscript revision and study supervision; all authors approved the final version of the manuscript.

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Abstract

BACKGROUND

mTOR gene is a key component of the PI3K/Akt/mTOR signaling pathway, and its dysregulation is associated with various diseases. Several studies have demonstrated that tea drinking is a protective factor against tuberculosis (TB). This study was designed to explore five single nucleotide polymorphisms (SNPs) of *mTOR* in the Han population of China to determine how their interactions with tea drinking affect susceptibility to TB.

AIM

To investigate if the polymorphisms of *mTOR* gene and the gene-tea interaction are associated with susceptibility to TB.

METHODS

In this case-control study, 503 patients with TB and 494 healthy controls were enrolled by a stratified sampling method. The cases were newly registered TB

reviewed and approved by the Medical Ethical Committee of Xiangya School of Public Health Central South University, No. XYGW-2018-11.

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patients from the county-level centers for disease control and prevention, and the healthy controls were permanent residents from Xin'ansi Community, Changsha city. Demographic data and environmental exposure information including tea drinking were obtained from the study participants. We genotyped five potentially functional SNP sites (rs2295080, rs2024627, rs1057079, rs12137958, and rs7525957) of *mTOR* gene and assessed their associations with the risk of TB using logistic regression analysis, and marginal structural linear odds models were used to estimate the gene-environment interactions.

RESULTS

The frequencies of four SNPs (rs2295080, rs2024627, rs1057079, and rs7525957) were found to be associated with susceptibility to TB ($P < 0.05$). Genotypes GT (OR 1.334), GG (OR 2.224), and GT + GG (OR 1.403) at rs2295080; genotypes CT (OR 1.562) and CT + TT (OR 1.578) at rs2024627, genotypes CT (OR 1.597), CC (OR 2.858), and CT + CC (OR 1.682) at rs1057079; and genotypes CT (OR 1.559) and CT + CC (OR 1.568) at rs7525957 of *mTOR* gene were significantly more prevalent in TB patients than in healthy controls. The relative excess risk of interaction between the four SNPs (rs2295080, rs2024627, rs1057079, and rs7525957) of *mTOR* genes and tea drinking were found to be -1.5187 (95%CI: -1.9826, -1.0547, $P < 0.05$), -1.8270 (95%CI: -2.3587, -1.2952, $P < 0.05$), -2.3246 (95%CI: -2.9417, -1.7076, $P < 0.05$) and -0.4235 (95%CI: -0.7756, -0.0714, $P < 0.05$), respectively, which suggest negative interactions.

CONCLUSION

The polymorphisms of *mTOR* (rs2295080, rs2024627, rs1057079, and rs7525957) are associated with susceptibility to TB, and there is a negative interaction between each of the four SNPs and tea drinking.

Key Words: Tuberculosis; *mTOR*; Tea drinking; Gene-environment interaction; The relative excess risk of interaction; Single nucleotide polymorphism

Core Tip: Our data demonstrated that genotypes GT, GG, and GT + GG at rs2295080; genotypes CT and CT + TT at rs2024627; genotypes CT, CC and CT + CC at rs1057079; and genotypes CT and CT + CC at rs7525957 of *mTOR* gene are associated with increased risk of tuberculosis in a Chinese population. In addition, there was a negative interaction between each of the four single nucleotide polymorphism (SNPs) and tea drinking. These findings may be helpful for identifying high-risk populations of tuberculosis, and suggest that promoting tea drinking might be a new way to reduce the risk of tuberculosis for individuals with mutations in the four SNPs.

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INTRODUCTION

Tuberculosis (TB) is a serious infectious disease caused by Mycobacterium tuberculosis (MTB) and remains one of the most serious challenges to global health. Approximately 10.0 million new TB cases and 1.451 million TB-associated deaths were reported worldwide in 2018[1]. The severity of national epidemics varies widely among countries. China is one of the 30 countries with the highest TB cases in the world, with an incidence rate of 61/100000[1]. Nearly one-quarter of the world's population is considered to be latently infected with MTB, whilst only 5%-15% of the infected individuals develop active TB in their lifetime[2]. TB was first discovered in different strains of mice and inbred rabbits, which developed different immune responses after

infection with MTB[3]. Since then, a series of studies including candidate gene screening, twin studies, family linkage analysis, and genome-wide association studies have shown that the incidence of TB varies among different races, ethnic groups, and families, indicating that host genetics influence TB susceptibility[4-6].

As one of the downstream effects of innate immune and adaptive immune pathways, autophagy can directly eliminate intracellular MTB, and it plays an indispensable role in the immune responses against MTB infection[7,8]. The formation of autophagy is regulated by a variety of signaling molecules, including the mechanistic target of rapamycin (mTOR), Beclin 1, Ca^{2+}, and p53. Among them, mTOR is at the center of various signaling pathways[9]. It is a serine/threonine protein kinase related to the PI3Ks, which senses fluctuations in intracellular and extracellular nutrients to modulate cellular growth, proliferation, metabolism, autophagy, and survival[10,11]. mTOR mainly achieves negative regulation of autophagy through Atg13. Phosphorylated Atg13 inhibits the formation of ULK-Atg13-FIP200 complexes, which is necessary for the formation of autophagosome, thereby inhibiting autophagy[12]. Moreover, under the action of various growth factors, PI3K/Akt can bind to the tyrosine protein kinase receptor to activate mTOR and inhibit autophagy[13].

Numerous environmental factors are associated with the risk of TB, and the protective effect of tea drinking has been established. Experimental and epidemiologic studies have demonstrated that there is a significantly negative association between tea drinking and TB. Tea polyphenols, especially epigallocatechin-3-gallate (EGCG), protect the immune system from various pathological processes including TB infection due to their antioxidant and free radical scavenging effects[14-16].

Recently, an experimental study confirmed that EGCG could effectively activate PI3K/Akt signaling, leading to the activation of mTOR and inhibition of autophagy[17]. Moreover, previous studies have suggested that polymorphisms of the *mTOR* gene are associated with susceptibility to various diseases[18-20]. Songane *et al*[21] observed no significant association between *mTOR* gene and TB in a multivariate analysis. However, it is still necessary to explore the relationship between *mTOR* gene and TB in other populations. In this case-control study, we investigated five single nucleotide polymorphisms (SNPs, rs2295080, rs2024627, rs1057079, rs12137958, and rs7525957) of *mTOR* in the Chinese Han population to clarify the role of *mTOR* polymorphism and the effect of their interactions with tea drinking on susceptibility to TB.

MATERIALS AND METHODS

Study population

This is a case-control study conducted in 2019. Sample size estimation was based on an estimated C allele of *mTOR* gene rs7525957 locus frequency of 10% (OR = 1.85, α = 0.05, two-sided, unpaired case-control design; and β = 0.15, two-sided). Based on the above assumptions, at least 490 subjects in each group were needed. A stratified sampling method was used to select cases and controls, and the details were reported in our previous publication[22]. The cases were newly registered TB patients from five randomly selected county-level centers for disease control and prevention (CDCs, Yueyang County CDC, Qidong County CDC, Hongjiang City CDC, Zixing City CDC, and Yueyanglou District CDC) from the 122 CDCs in Hunan Province. Healthy controls were selected from the permanent residents in Xin'ansi Community (a community in Changsha city) using a gender-age frequency matching method. Included criteria for cases and controls were strictly in accordance with the standards that have been previously described[22]. The study protocol was approved by the Medical Ethics Committee of Xiangya School of Public Health, Central South University, No. XYGW-2018-11. The research was carried out in strict accordance with the protocol, and all the included participants (> 18 years old) provided written informed consent.

Information and sample collection

Each participant completed a self-administered questionnaire on baseline characteristics and lifestyle, which included information on sex, age, height, weight, education, marital status, smoking status, tea drinking, alcohol consumption, and Bacillus Calmette–Guérin (BCG) vaccination. All the questionnaires were completed by trained research staffs in accordance with the instructions. Blood sample (5 mL) was collected from each participant by certified nurses in EDTA anticoagulant tube and stored at 4 °C immediately.

Selection of SNPs and genotyping

In this study, candidate SNP sites of *mTOR* were collected based on the following two points: Firstly, we selected the SNP sites previously associated with susceptibility to TB on PubMed, and secondly, and the SNP sites associated with other infectious diseases. In addition, only loci with a minor allele frequency of at least 5% were included to ensure the statistical efficacy of this study. Using NCBI SNP database (http://www.ncbi.nlm.nih.gov), we searched and learned about the frequencies of corresponding SNP sites of *mTOR* gene. Finally, five SNPs including rs2295080, rs2024627, rs1057079, rs12137958, and rs7525957 of *mTOR* gene were selected for analysis in this study.

A Wizard Genomic DNA purification kit (Promega) was used to extract the peripheral white blood cell genome, and the quality-controlled DNA was frozen at -20 °C upon collection. The site sequences of rs2295080, rs2024627, rs1057079, rs12137958, and rs7525957 of *mTOR* gene were identified in the GenBank and Assay Design 3.1 (Sequenom) was used to design the appropriate primers. The synthesized primers were subjected to quality assessment by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF). The polymerase chain reaction (PCR) volume was 5 μL, which included 1.8 μL distilled water (ddH_2O), 0.5 μL 10 × PCR buffer, 0.4 μL $MgCl_2$ (25 mmol/L), 0.1 μL dNTP (25 mmol/L), 0.2 μL Hotstar, 1 μL PCR primer mix, and 1 μL gDNA (20-50 ng). The reaction condition was 95 °C predegeneration for 2 min, amplification (95 °C for 30 s, 56 °C for 30 s, and 72 °C for 60 s) for 45 cycles, and 72 °C extension for 5 min. The enzyme digestion reaction system , included 1.53 μL ddH_2O, 0.17 μL SAP buffer, and 0.3 μL SAP enzyme; the reaction condition was 37 °C for 40 min and 85 °C for 5 min. Single base extension reaction system was 2 μL, which included 0.619 μL ddH_2O, 0.2 μL iplex buffer, 0.2 μL terminator mix, 0.94 μL extension primer mix, and 0.041 μL iplex enzyme; the corresponding reaction condition was 94 °C predegeneration for 30 s, 40 cycles of amplification (five cycles of three temperature settings: 94 °C for 5 s, 52 °C for 5 s, 80 °C for 5 s, and 72 °C extension for 3 min). Subsequently, resin was purified by plating clean resin on a 6-mg resin plate, and the resin-extended product was transferred to a 384-well SpectroCHIP (Sequenom) chip for spotting (MassARRAY Nanodispenser RS1000). Sequenom MassARRAY® SNP assay was used to determine the difference in bases caused by SNP polymorphism as molecular weight difference. MALDI-TOF was used to detect the molecular weight of the extension product, and the analysis was performed using MassArray TYPER 4.0. SNP typing was determined by the difference in molecular weight.

Statistical analysis

All the statistical analyses were performed using SPSS 23.0 software (SPSS Inc., Chicago, IL, United States). Continuous variables were presented as mean ± SD and categorical variables were presented as proportions. The independent-sample *t* test was used for the analysis of continuous variables. The Chi-square test (χ^2) was conducted for the comparison of categorical data and Hardy-Weinberg equilibrium detection. Odds ratios (ORs) and the corresponding 95%CIs were calculated to measure the association between each SNP and TB susceptibility using an unconditional logistic regression model, with the adjustment of possible confounders, such as age and sex. The interaction of additive effects between SNP and tea drinking was analyzed and the relative excess risk of interaction (RERI) was used to estimate if the main effect on TB was meaningful. Point estimation and interval estimation of RERI were calculated using Marginal Structural Linear Odds Models[23]. RERI > 0 indicates positive interactions. All statistical tests were two-sided and a P value < 0.05 indicated statistical significance.

Biostatistics statement

The statistical methods of this study were reviewed by Meng-Shi Chen from the Department of Epidemiology and Health Statistics, Xiangya School of Public Health, Central South University.

RESULTS

A total of 503 TB patients and 494 healthy controls were included in the study. There was no statistically significant difference (P > 0.05) in terms of sex, age, marital status, educational background, and alcohol consumption between the two groups. Differences in body mass index (BMI), history of BCG vaccination, smoking status, and

tea drinking were statistically significant ($P < 0.05$) (Table 1).

The distribution of SNPs at the selected five sites of the *mTOR* gene in each group is shown in Table 2. The genotypic distributions in TB patients and healthy controls were tested separately for Hardy-Weinberg equilibrium. No significant deviation was observed with all the five polymorphism sites ($P > 0.05$).

The univariate analysis showed that the genotypes of rs2295080 ($\chi^2 = 9.621$, $P < 0.05$), genotypes of rs2024627 ($\chi^2 = 6.989$, $P < 0.05$), genotypes of rs1057079 ($\chi^2 = 19.708$, $P < 0.001$), and genotypes of rs7525957 ($\chi^2 = 9.568$, $P < 0.05$) were closely associated with TB incidence. In addition, no statistically significant difference was observed in the genotypes of rs12137958 between the two groups ($P > 0.05$). Genotype TT at rs2295080, genotype CC at rs2024627, genotype TT at rs1057079, and genotype TT at rs7525957 were all less prevalent in TB patients. Moreover, genotype TT at rs2024627 and genotype AA at rs12137958 were rare in the participants (Table 2).

Further multivariate unconditional logistic regression analysis confirmed that rs2295080, rs2024627, rs1057079, and rs7525957 of *mTOR* gene were associated with susceptibility to TB. Genotypes GT, GG, and GT + GG at rs2295080; genotypes CT and CT + TT at rs2024627; genotypes CT, CC, and CT + CC at rs1057079; and genotypes CT and CT + CC at rs7525957 of *mTOR* gene were significantly more prevalent in TB patients than in healthy controls (Table 3).

Marginal structural linear odds models were used to examine the interactions between the selected five sites of the *mTOR* gene and tea drinking. After adjusting for the covariates of sex, age, marital status, educational background, BMI, alcohol drinking, smoking status, and history of BCG vaccination, the RERI between rs2295080 of *mTOR* genes and tea drinking was -1.5187, which suggests negative interactions (Table 4). Similarly, negative interactions were also observed between each of the other three sites (rs2024627, rs1057079 and rs7525957) of the *mTOR* gene and tea drinking, with the adjusted RERI of -1.8270, -2.3246, and -0.4235, respectively (Table 4).

DISCUSSION

This case-control study examined the association between *mTOR* polymorphisms and TB susceptibility as well as their interactions with tea drinking. It is noteworthy that the frequencies of four SNPs (rs2295080, rs2024627, rs1057079, and rs7525957) of *mTOR* gene were associated with susceptibility to TB. However, no association was observed between the genotypes of rs12137958 and TB incidence.

mTOR gene is a key component of the PI3K/Akt/mTOR signaling pathway, and its dysregulation is associated with the pathogenesis and progression of various cancers[20,24]. Although the reason for this anomaly is still controversial, it is biologically plausible that functional SNPs of the *mTOR* gene may contribute to cancer susceptibility[25]. *In vitro* studies have revealed a higher transcription activity of *mTOR* in the presence of rs2295080 T allele in 786-O, HEK293, GES-1, and HeLa cell lines. Similarly, individuals with TT genotypes have higher *mTOR* mRNA level[18,26]. These indicate that the rs2295080 T allele could probably increase the affinity of special transcription factors to the *mTOR* promoter region and subsequently contribute to the enhanced mTOR activity in humans[26]. Multiple population studies have confirmed that *mTOR* polymorphisms affect the susceptibility of various kinds of cancers. In previous studies, individuals with TG/GG genotype displayed a significantly decreased susceptibility to gastric cancer, colorectal cancer, and breast cancer, compared with those carrying rs2295080 TT genotype[26-28]. For SNP rs1057079, the G allele carriers are at higher risk of developing esophageal squamous cell carcinoma, colon cancer, and breast cancer[29-31]. In addition, rs2024627, located in the intron, affects the expression of *mTOR* gene[32], and it is recommended as a genetic marker of pharmacogenetics of kidney transplant[33]. Our results showed that the frequencies of our SNPs (including rs2295080, rs1057079, and rs2024627) of *mTOR* gene were associated with TB susceptibility, suggesting that these functional SNPs of *mTOR* gene might play a critical role in the prediction of susceptibility to TB.

To our knowledge, no report has been made on the association of rs12137958 or rs7525957 of *mTOR* gene with TB susceptibility. Although we speculate that these SNPs with unknown functional effects on exons or introns may affect the binding capacity of transcription factors and subsequent gene transcription, an explanation for the correlation should be determined in future mechanistic biological studies. Several studies have demonstrated that mTOR targeted therapies could be designed to block the induction of the prosurvival, proliferative, and oncogenic functions of mTOR[34]. Therefore, this finding is important not only for the understanding of the pathogenesis

Table 1 Demographic characteristics and associated risk factors in tuberculosis patients *vs*. healthy controls

	TB patients (*n* = 503)		Healthy controls (*n* = 494)		χ^2	*P* value
	n	%	*n*	%		
Sex						
Male	369	73.36	348	70.45	1.048	0.306
Female	134	26.64	146	29.55		
Age, yr						
18-30	68	13.52	78	15.79	1.908	0.592
31-50	190	37.77	178	36.03		
51-70	167	32.20	153	30.97		
> 70	78	15.51	85	17.21		
Marital status						
Married	345	68.59	330	66.80	0.364	0.546
Other	158	31.41	164	33.20		
Educational background						
Primary school or below	198	39.36	220	44.53	2.824	0.244
Junior high school	161	32.01	148	29.96		
Senior high school or above	144	28.63	126	25.51		
BMI, kg/m^2						
< 18.5	180	35.79	169	34.21	9.310	0.010[a]
18.5-24.9	297	59.05	274	55.47		
≥ 25.0	26	5.17	51	10.32		
History of BCG vaccination						
Yes	105	20.87	141	28.54	7.884	0.005[a]
No	398	79.13	353	71.46		
Smoking						
Yes	298	59.24	240	48.58	11.403	< 0.001[a]
No	205	40.76	254	51.42		
Alcohol drinking						
Yes	91	18.09	77	15.59	1.116	0.291
No	412	81.91	417	84.41		
Tea drinking						
Yes	247	49.11	293	59.31	10.457	0.001[a]
No	256	50.89	201	40.69		

[a]*P* < 0.05. TB: Tuberculosis; BMI: Body mass index; BCG: Bacillus Calmette–Guérin.

of TB, but also for the identification of high-risk populations of TB and the development of appropriate population-specific prevention measures to control the spread of TB.

Moreover, marginal structural linear odds model analysis showed that there were negative interactions between rs2295080, rs2024627, rs1057079, and rs7525957 of *mTOR* genes and tea drinking, which suggests that when mutations occur in these four SNPs of *mTOR* genes, the risk for TB will decrease for individuals that drink tea regularly. Additionally, our previous study confirmed that increasing tea consumption is associated with a decreased risk of TB[15]. Moreover, some studies have demonstrated that catechin, an important antioxidant extracted from tea leaves, can protect cells

Table 2 Genotypes of the *mTOR* gene in the two groups

		TB patients		Healthy controls		χ^2	*P* value
		n	%	*n*	%		
rs2295080	TT	286	56.86	323	65.38	9.621	0.008[a]
	GT	191	37.97	158	31.98		
	GG	26	5.17	13	2.63		
HWE-*P*				0.220			
rs2024627	CC	406	80.72	429	86.84	6.989	0.030[a]
	CT	93	18.49	63	12.75		
	TT	4	0.80	2	0.40		
HWE-*P*				0.847			
rs1057079	TT	271	53.88	330	66.80	19.708	< 0.001[a]
	CT	204	40.56	152	30.77		
	CC	28	5.57	12	2.43		
HWE-*P*				0.259			
rs12137958	GG	414	82.31	419	84.82	1.626	0.526
	AG	87	17.30	72	14.57		
	AA	2	0.40	3	0.61		
HWE-*P*				0.961			
rs7525957	TT	368	73.16	402	81.38	9.568	0.008[a]
	CT	126	25.05	86	17.41		
	CC	9	1.79	6	1.21		
HWE-*P*				0.566			

[a]*P* < 0.05. TB: Tuberculosis; HWE-*P*: Hardy-Weinberg equilibrium-*P* value.

from damage by inducing antioxidant enzymes, inhibiting oxidase, and scavenging free radicals[14,16]. EGCG, which is the most potent component in catechins, plays an important role in arresting the growth of tubercle bacillus. Previous studies demonstrated that EGCG could inhibit the transcription of *tryptophan-aspartate containing coat protein* (*TACO*) gene, which is essential in the entry and intracellular survival of mycobacteria[35]. Anand *et al*[16] found that EGCG could inhibit Sp1 transcription factor, a DNA-binding protein located in the promoter region of *TACO* gene, and block the binding of Sp1 binding sequence to the promoter region of fatty acid synthase gene, thus down-regulating the expression of *TACO* gene. Genetic mutations cannot be altered, and hence, our findings suggest that promoting tea drinking may be considered a new way to reduce the risk of developing TB for individuals with mutations in these four SNPs.

This study had some limitations. Firstly, TB is a complex disease, and the genetic background of the study population or the difference in environmental exposure led to an inevitable heterogeneity between studies and a limited control of confounding factors in the study. Hence, the analyses of gene-gene, gene-environment, and environment-environment interactions are required. Secondly, only five potentially functional SNPs of *mTOR* gene were investigated in the present study, which did not cover all the variants in *mTOR* gene, and the importance of combining SNPs was neglected. Hence, the results could not fully represent the role of mTOR genetic susceptibility factors in TB. A joint analysis of multiple genes or multiple sites of the same gene will facilitate the discovery of true positive associations and the elucidation of the mechanism of these genetic variants.

Table 3 *mTOR* gene polymorphism *vs*. tuberculosis incidence

		TB patients		Healthy controls		OR_c (95%CI)	OR_a[1] (95%CI)
		n	%	*n*	%		
rs2295080	TT	286	56.86	323	65.38	1	1
	GT	191	37.97	158	31.98	1.365 (1.048-1.778)	1.334 (1.018-1.749)[a]
	GG	26	5.17	13	2.63	2.259 (1.139-4.479)	2.224 (1.110-4.458)[a]
	GT + GG	217	43.14	171	34.61	1.433 (1.110-1.851)	1.403 (1.080-1.823)[a]
rs2024627	CC	406	80.72	429	86.84	1	1
	CT	93	18.49	63	12.75	1.560 (1.102-2.208)	1.562 (1.096-2.226)[a]
	TT	4	0.80	2	0.40	2.113 (0.385-11.600)	2.069 (0.369-11.607)
	CT + TT	97	19.29	65	13.15	1.577 (1.120-2.220)	1.578 (1.113-2.237)[a]
rs1057079	TT	271	53.88	330	66.80	1	1
	CT	204	40.56	152	30.77	1.634 (1.255-2.129)	1.597 (1.216-2.097)[a]
	CC	28	5.57	12	2.43	2.841 (1.418-5.694)	2.858 (1.404-5.818)[a]
	CT + CC	232	46.13	164	33.20	1.723 (1.333-2.226)	1.682 (1.290-2.194)[a]
rs12137958	GG	414	82.31	419	84.82	1	1
	AG	87	17.30	72	14.57	1.223 (0.870-1.719)	1.187 (0.840-1.678)
	AA	2	0.40	3	0.61	0.675 (0.112-4.059)	0.879 (0.144-5.379)
	AG + AA	89	17.70	75	15.18	1.201 (0.858-1.680)	1.176 (0.836-1.656)
rs7525957	TT	368	73.16	402	81.38	1	1
	CT	126	25.05	86	17.41	1.600 (1.176-2.179)	1.559 (1.138-2.136)[a]
	CC	9	1.79	6	1.21	1.639 (0.578-4.648)	1.686 (0.588-4.831)
	CT + CC	135	26.84	92	18.62	1.603 (1.187-2.165)	1.568 (1.154-2.130)[a]

[a]$P < 0.05$.

[1]Adjusted for the covariates of sex, age, marital status, educational background, body mass index, smoking status, alcohol drinking, tea drinking and Bacillus Calmette–Guérin vaccination. TB: Tuberculosis.

CONCLUSION

The present study provides evidence of the association between *mTOR* polymorphism and TB susceptibility. Rs2295080, rs2024627, rs1057079, and rs7525957 of *mTOR* gene were associated with significant increased risk of TB in a Chinese population. In addition, there was a negative interaction between each of the four SNPs and tea drinking. Nevertheless, these findings should be verified by larger independent population-based studies.

Table 4 Impact of interactions between genotypes of *mTOR* gene and tea drinking on incidence of tuberculosis

		Tea drinking		$RERI_c$	$RERI_a$[1] (95%CI)
		No	Yes		
rs2295080	TT	245	364	-2.0341	-1.5187 (-1.9826, -1.0547)[a]
	GT + GG	212	176		
rs2024627	CC	367	468	-1.6561	-1.8270 (-2.3587, -1.2952)[a]
	CT + TT	90	72		
rs1057079	TT	228	373	-2.7054	-2.3246 (-2.9417, -1.7076)[a]
	CT + CC	229	167		
rs7525957	TT	326	444	-1.3684	-0.4235 (-0.7756, -0.0714)[a]
	CT + CC	131	96		

[1]Adjusted for the covariates of sex, age, marital status, educational background, body mass index, smoking status, alcohol drinking and Bacillus Calmette–Guérin vaccination.

[a]$P < 0.05$, RERI < 0 suggests negative interactions. RERI: Relative excess risk of interaction.

ARTICLE HIGHLIGHTS

Research background

Tuberculosis (TB) is a serious infectious disease caused by Mycobacterium tuberculosis. The incidence of TB has been shown to vary among different races, ethnic groups, and families, indicating that host genetics influence TB susceptibility. *mTOR* gene is a key component of the PI3K/Akt/mTOR signaling pathway, and its dysregulation is associated with various diseases. In addition, several studies have demonstrated that tea is a protective factor against TB due to its antioxidant and free radical scavenging effects.

Research motivation

Investigations have suggested that polymorphisms of the *mTOR* gene are associated with susceptibility to various diseases. And epigallocatechin-3-gallate, the major component of tea catechins, could effectively activate PI3K/Akt signaling, leading to the activation of mTOR and inhibition of autophagy. The role of *mTOR* polymorphisms in TB is still inconclusive. Moreover, whether there is any interaction on TB risk between tea drinking and polymorphisms of *mTOR* gene has not been reported.

Research objectives

This study aimed to investigate five single nucleotide polymorphisms (SNPs) of *mTOR* in the Han population of China to determine how their interactions with tea drinking affect susceptibility to TB.

Research methods

In this case-control study, 503 TB patients and 494 healthy controls were enrolled by a stratified sampling method. The cases were newly registered TB patients from the county-level centers for disease control and prevention, and the healthy controls were permanent residents from Xin'ansi Community, Changsha city. Demographic data and environmental exposure information including tea drinking were obtained from the study participants. We genotyped five potentially functional SNP sites (rs2295080, rs2024627, rs1057079, rs12137958, and rs7525957) of *mTOR* gene and assessed their associations with the risk of TB using logistic regression analysis, and marginal structural linear odds models were used to estimate the gene-environment interactions.

Research results

The frequencies of four SNPs (rs2295080, rs2024627, rs1057079, and rs7525957) were found to be associated with susceptibility to TB ($P < 0.05$). Genotypes GT (OR 1.334), GG (OR 2.224), and GT + GG (OR 1.403) at rs2295080; genotypes CT (OR 1.562) and CT

+ TT (OR 1.578) at rs2024627, genotypes CT (OR 1.597), CC (OR 2.858), and CT + CC (OR 1.682) at rs1057079; and genotypes CT (OR 1.559) and CT + CC (OR 1.568) at rs7525957 of *mTOR* gene were significantly more prevalent in TB patients than in healthy controls. The relative excess risk of interaction between the four SNPs of *mTOR* genes and tea drinking was found to be -1.5187 (95%CI -1.9826, -1.0547, $P < 0.05$), -1.8270 (95%CI -2.3587, -1.2952, $P < 0.05$), -2.3246 (95%CI -2.9417, -1.7076, $P < 0.05$) and -0.4235 (95%CI -0.7756, -0.0714, $P < 0.05$), respectively, which suggest negative interactions.

Research conclusions

The polymorphisms of *mTOR* (rs2295080, rs2024627, rs1057079, and rs7525957) are associated with susceptibility to TB, and there is a negative interaction between each of the four SNPs and tea drinking. These findings are significant for identifying populations with high risk of developing TB, and suggest that preventive measures through promoting the consumption of tea should be emphasized in the high-risk populations.

Research perspectives

Since TB is a complex disease involving various factors including heredity, biology and environment, the genetic background of the study population or the difference in environmental exposure may lead to an inevitable heterogeneity between studies. Hence, larger independent population-based studies in different countries or ethnic groups are required to validate our initial findings.

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REFERENCES

1 **World Health Organization**. Global tuberculosis report 2019. Geneva: WHO, 2019. [accessed 2020; February 2] Available from: https://apps.who.int/iris/bitstream/handle/10665/329368/9789241565714-eng.pdf
2 **Ganmaa D**, Uyanga B, Zhou X, Gantsetseg G, Delgerekh B, Enkhmaa D, Khulan D, Ariunzaya S, Sumiya E, Bolortuya B, Yanjmaa J, Enkhtsetseg T, Munkhzaya A, Tunsag M, Khudyakov P, Seddon JA, Marais BJ, Batbayar O, Erdenetuya G, Amarsaikhan B, Spiegelman D, Tsolmon J, Martineau AR. Vitamin D Supplements for Prevention of Tuberculosis Infection and Disease. *N Engl J Med* 2020; **383**: 359-368 [PMID: 32706534 DOI: 10.1056/NEJMoa1915176]
3 **Smith CM**, Sassetti CM. Modeling Diversity: Do Homogeneous Laboratory Strains Limit Discovery? *Trends Microbiol* 2018; **26**: 892-895 [PMID: 30166218 DOI: 10.1016/j.tim.2018.08.002]
4 **Aravindan PP**. Host genetics and tuberculosis: Theory of genetic polymorphism and tuberculosis. *Lung India* 2019; **36**: 244-252 [PMID: 31031349 DOI: 10.4103/lungindia.lungindia_146_15]
5 **Lawn SD**, Zumla AI. Tuberculosis. *Lancet* 2011; **378**: 57-72 [PMID: 21420161 DOI: 10.1016/S0140-6736(10)62173-3]
6 **Dallmann-Sauer M**, Correa-Macedo W, Schurr E. Human genetics of mycobacterial disease. *Mamm Genome* 2018; **29**: 523-538 [PMID: 30116885 DOI: 10.1007/s00335-018-9765-4]
7 **Wu YW**, Li F. Bacterial interaction with host autophagy. *Virulence* 2019; **10**: 352-362 [PMID: 30978154 DOI: 10.1080/21505594.2019.1602020]
8 **Xiao Y**, Cai W. Autophagy and Bacterial Infection. *Adv Exp Med Biol* 2020; **1207**: 413-423 [PMID: 32671764 DOI: 10.1007/978-981-15-4272-5_29]
9 **Yang Z**, Klionsky DJ. Mammalian autophagy: core molecular machinery and signaling regulation. *Curr Opin Cell Biol* 2010; **22**: 124-131 [PMID: 20034776 DOI: 10.1016/j.ceb.2009.11.014]
10 **Liu C**, Chapman NM, Karmaus PW, Zeng H, Chi H. mTOR and metabolic regulation of conventional and regulatory T cells. *J Leukoc Biol* 2015; **97**: 837-847 [PMID: 25714803 DOI: 10.1189/jlb.2RI0814-408R]
11 **Rabanal-Ruiz Y**, Otten EG, Korolchuk VI. mTORC1 as the main gateway to autophagy. *Essays Biochem* 2017; **61**: 565-584 [PMID: 29233869 DOI: 10.1042/EBC20170027]
12 **Jung CH**, Jun CB, Ro SH, Kim YM, Otto NM, Cao J, Kundu M, Kim DH. ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. *Mol Biol Cell* 2009; **20**: 1992-2003 [PMID: 19225151 DOI: 10.1091/mbc.E08-12-1249]
13 **Mizushima N**, Klionsky DJ. Protein turnover via autophagy: implications for metabolism. *Annu Rev Nutr* 2007; **27**: 19-40 [PMID: 17311494 DOI: 10.1146/annurev.nutr.27.061406.093749]
14 **Maiolini M**, Gause S, Taylor J, Steakin T, Shipp G, Lamichhane P, Deshmukh B, Shinde V, Bishayee A, Deshmukh RR. The War against Tuberculosis: A Review of Natural Compounds and Their Derivatives. *Molecules* 2020; 25 [PMID: 32630150 DOI: 10.3390/molecules25133011]
15 **Chen M**, Deng J, Li W, Lin D, Su C, Wang M, Li X, Abuaku BK, Tan H, Wen SW. Impact of tea drinking

upon tuberculosis: a neglected issue. *BMC Public Health* 2015; **15**: 515 [PMID: 26021567 DOI: 10.1186/s12889-015-1855-6]
16 **Anand PK**, Kaul D, Sharma M. Green tea polyphenol inhibits Mycobacterium tuberculosis survival within human macrophages. *Int J Biochem Cell Biol* 2006; **38**: 600-609 [PMID: 16352457 DOI: 10.1016/j.biocel.2005.10.021]
17 **Ding ML**, Ma H, Man YG, Lv HY. Protective effects of a green tea polyphenol, epigallocatechin-3-gallate, against sevoflurane-induced neuronal apoptosis involve regulation of CREB/BDNF/TrkB and PI3K/Akt/mTOR signalling pathways in neonatal mice. *Can J Physiol Pharmacol* 2017; **95**: 1396-1405 [PMID: 28679060 DOI: 10.1139/cjpp-2016-0333]
18 **Cao Q**, Ju X, Li P, Meng X, Shao P, Cai H, Wang M, Zhang Z, Qin C, Yin C. A functional variant in the MTOR promoter modulates its expression and is associated with renal cell cancer risk. *PLoS One* 2012; **7**: e50302 [PMID: 23209702 DOI: 10.1371/journal.pone.0050302]
19 **Bonnet S**, Falkowski S, Deppenweiler M, Monchaud C, Arnion H, Picard N, Woillard JB. Effect of genetic polymorphisms in CYP3A4, CYP3A5, and m-TOR on everolimus blood exposure and clinical outcomes in cancer patients. *Pharmacogenomics J* 2020 [PMID: 32015456 DOI: 10.1038/s41397-020-0152-7]
20 **Zining J**, Lu X, Caiyun H, Yuan Y. Genetic polymorphisms of mTOR and cancer risk: a systematic review and updated meta-analysis. *Oncotarget* 2016; **7**: 57464-57480 [PMID: 27462867 DOI: 10.18632/oncotarget.10805]
21 **Songane M**, Kleinnijenhuis J, Alisjahbana B, Sahiratmadja E, Parwati I, Oosting M, Plantinga TS, Joosten LA, Netea MG, Ottenhoff TH, van de Vosse E, van Crevel R. Polymorphisms in autophagy genes and susceptibility to tuberculosis. *PLoS One* 2012; **7**: e41618 [PMID: 22879892 DOI: 10.1371/journal.pone.0041618]
22 **Chen M**, Liang Y, Li W, Wang M, Hu L, Abuaku BK, Huang X, Tan H, Wen SW. Impact of MBL and MASP-2 gene polymorphism and its interaction on susceptibility to tuberculosis. *BMC Infect Dis* 2015; **15**: 151 [PMID: 25887173 DOI: 10.1186/s12879-015-0879-y]
23 **VanderWeele TJ**, Vansteelandt S. A weighting approach to causal effects and additive interaction in case-control studies: marginal structural linear odds models. *Am J Epidemiol* 2011; **174**: 1197-1203 [PMID: 22058231 DOI: 10.1093/aje/kwr334]
24 **Wang FM**, Zhang X, Lan L, Ji JM, Tang HB, Yao XJ, Jiang Y, Qian J, Xu XG, Li Q, Yao P, Li JH, Shen YP. [Association of PD-1, TIM-3 and TREM-1 single nucleotide polymorphisms with pulmonary tuberculosis susceptibility]. *Zhonghua Yi Xue Za Zhi* 2017; **97**: 3301-3305 [PMID: 29141374 DOI: 10.3760/cma.j.issn.0376-2491.2017.42.006]
25 **Qi GH**, Wang CH, Zhang HG, Yu JG, Ding F, Song ZC, Xia QH. Comprehensive analysis of the effect of rs2295080 and rs2536 polymorphisms within the mTOR gene on cancer risk. *Biosci Rep* 2020; 40 [PMID: 32597485 DOI: 10.1042/BSR20191825]
26 **Xu M**, Tao G, Kang M, Gao Y, Zhu H, Gong W, Wang M, Wu D, Zhang Z, Zhao Q. A polymorphism (rs2295080) in mTOR promoter region and its association with gastric cancer in a Chinese population. *PLoS One* 2013; **8**: e60080 [PMID: 23555892 DOI: 10.1371/journal.pone.0060080]
27 **Xu M**, Gao Y, Yu T, Wang J, Cheng L, Cheng L, Cheng D, Zhu B. Functional promoter rs2295080 T>G variant in MTOR gene is associated with risk of colorectal cancer in a Chinese population. *Biomed Pharmacother* 2015; **70**: 28-32 [PMID: 25776475 DOI: 10.1016/j.biopha.2014.12.045]
28 **Zhao Y**, Diao Y, Wang X, Lin S, Wang M, Kang H, Yang P, Dai C, Liu X, Liu K, Li S, Zhu Y, Dai Z. Impacts of the mTOR gene polymorphisms rs2536 and rs2295080 on breast cancer risk in the Chinese population. *Oncotarget* 2016; **7**: 58174-58180 [PMID: 27533457 DOI: 10.18632/oncotarget.11272]
29 **Zhu J**, Wang M, Zhu M, He J, Wang JC, Jin L, Wang XF, Xiang JQ, Wei Q. Associations of PI3KR1 and mTOR polymorphisms with esophageal squamous cell carcinoma risk and gene-environment interactions in Eastern Chinese populations. *Sci Rep* 2015; **5**: 8250 [PMID: 25654238 DOI: 10.1038/srep08250]
30 **Slattery ML**, Herrick JS, Lundgreen A, Fitzpatrick FA, Curtin K, Wolff RK. Genetic variation in a metabolic signaling pathway and colon and rectal cancer risk: mTOR, PTEN, STK11, RPKAA1, PRKAG2, TSC1, TSC2, PI3K and Akt1. *Carcinogenesis* 2010; **31**: 1604-1611 [PMID: 20622004 DOI: 10.1093/carcin/bgq142]
31 **Slattery ML**, John EM, Torres-Mejia G, Lundgreen A, Herrick JS, Baumgartner KB, Hines LM, Stern MC, Wolff RK. Genetic variation in genes involved in hormones, inflammation and energetic factors and breast cancer risk in an admixed population. *Carcinogenesis* 2012; **33**: 1512-1521 [PMID: 22562547 DOI: 10.1093/carcin/bgs163]
32 **Slattery ML**, Lundgreen A, Mullany LE, Penney RB, Wolff RK. Influence of CHIEF pathway genes on gene expression: a pathway approach to functionality. *Int J Mol Epidemiol Genet* 2014; **5**: 100-111 [PMID: 24959314]
33 **Pouché L**, Stojanova J, Marquet P, Picard N. New challenges and promises in solid organ transplantation pharmacogenetics: the genetic variability of proteins involved in the pharmacodynamics of immunosuppressive drugs. *Pharmacogenomics* 2016; **17**: 277-296 [PMID: 26799749 DOI: 10.2217/pgs.15.169]
34 **Harwood FC**, Klein Geltink RI, O'Hara BP, Cardone M, Janke L, Finkelstein D, Entin I, Paul L, Houghton PJ, Grosveld GC. ETV7 is an essential component of a rapamycin-insensitive mTOR complex in cancer. *Sci Adv* 2018; **4**: eaar3938 [PMID: 30258985 DOI: 10.1126/sciadv.aar3938]
35 **Anand PK**, Kaul D. Downregulation of TACO gene transcription restricts mycobacterial entry/survival within human macrophages. *FEMS Microbiol Lett* 2005; **250**: 137-144 [PMID: 16040207 DOI: 10.1016/j.femsle.2005.06.056]

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